Hormonal regulation of the insulin-responsive glucose transporter, GLUT4: some recent advances

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Régulation hormonale du transporteur du glucose répondant à l'insuline, GLUT4: nouvelles avancées

RÉSUMÉ

A ce jour, quatre transporteurs facilitants du glucose ont été clônés et caractérisés dans leur fonction. Ces protéines qui sont les produits de gènes distincts, s'expriment spécifiquement selon le tissu, et catalysent le mouvement facilitant du glucose dans son gradient chimique à travers la membrane de la cellule. Il est possible de proposer une explication à cette diversité d'expression basée sur l'analyse des propriétés cinétiques et fonctionnelles de ces différents isoformes. Ainsi, on pense que GLUT1 est impliqué dans le transport du glucose à travers les barrières entre le sang et les organes tels que la rétine, et à travers la barrière entre le sang et le cerveau; il est aussi impliqué dans le maintien de niveaux basals ou d'entretien de l'activité de transport du glucose dans beaucoup de types de cellules, sinon dans tous. GLUT2 est un transporteur à haute capacité et à faible affinité qui convient idéalement à cet isoforme dans son rôle dans les flux d'entrée et de sortie du foie; de plus, il joue potentiellement un rôle-clé dans la reconnaissance du glucose dans le pancréas. GLUT 3 est un transporteur à haute affinité qui s'exprime essentiellement dans le cerveau et les nerfs, qui dépendent presqu'entièrement du glucose pour leur énergie. Ainsi, on peut postuler la présence d'un transporteur à haute affinité dans ces cellules pour leur conférer la capacité de rechercher le glucose avec efficacité, même dans des conditions d'hypoglycémie. Nous présentons ici les nouvelles avancées dans les connaisances de la régulation du dit transporteur du glucose répondant à l'insuline, GLUT4, dont les principaux sites d'expression sont le muscle, le coeur et le tissu adipeux.

To date, four facilitative glucose transporters (GLUT1–GLUT4) have been cloned and functionally characterized. These proteins are the products of distinct genes, are expressed in a tissue-specific fashion (see Table 1), and catalyse the facilitative movement of glucose down its chemical gradient across the cell membrane. The transport is driven only by the chemical gradient and is not coupled to either Na or proton gradients or the hydrolysis of ATP. Details of the kinetics, substrate specificity and structure have been extensively reviewed in recent times, and so the reader is

Table 1. *Major sites of expression of the different glucose transporters*

Isoform	Tissue*	K_m for deoxyglucose (mM)	
		Mean	SD
GLUT1	Placenta, brain, blood–tissue barrier, adipose and muscle tissue (low levels), tissue culture cells, transformed cells	6·9	1·5
	Galactose (K_m 17 mM)†		
GLUT2	Liver, pancreatic β-cell, kidney proximal tubule and small intestine (basolateral membranes)	11·2	1·1
	Fructose (K_m 66 mM)†		
GLUT3	Brain and nerve cells in rodents Brain and nerve, low levels in placenta, kidney, liver and heart (humans)	1·4	0·06
	Galactose (K_m 8·5 mM)†		
GLUT4	Muscle, heart and adipose tissue	4·6	0·3
GLUT5‡	Small intestine (apical membranes), brain, muscle and adipose tissue, muscle and brain at low levels	n/a	
	Fructose (K_m 6 mM)†		
GLUT7	Microsomal glucose transporter, liver		

* Major sites of expression of the different facilitative glucose transporter isoforms.
† Approximate K_m values for alternative substrates.
‡ GLUT5 is exclusively a fructose transporter.

referred to one of a number of excellent reviews for further background information (for example, Mueckler, 1990; Baldwin, 1993; Bell *et al.* 1993; Gould & Holman, 1993). It is possible to propose a rationale for this diversity of expression based on analysis of the kinetic and functional properties of these different isoforms (Bell *et al.* 1993; Gould & Holman, 1993). Thus, GLUT1 is thought to be involved in the transport of glucose across blood–tissue barriers such as the retina and blood–brain barrier, and is also involved in maintaining basal or 'house-keeping' levels of transport activity in many, if not all, cell types. GLUT2 is a high-capacity low-affinity transporter which ideally suits this isoform to its role in glucose influx and efflux from the liver, and potentially plays a key role in glucose-sensing in the pancreas. GLUT3 is a high-affinity transporter expressed mainly in brain and nerve, which depend almost entirely on glucose for their energy. Thus, the presence of a high-affinity transporter in these cells may be postulated to endow them with the ability to effectively scavenge for glucose, even under conditions of low blood glucose. Two other members of the family shown in Table 1 deserve some comment. A fructose transporter isoform, GLUT5, has recently been identified (Burant *et al.* 1992). This is the subject of review by Brot-Laroche (1996), and so is not discussed further here. Finally, another member of this family was identified in 1992 and designated GLUT7 (Waddell *et al.* 1992). This transporter purportedly is involved in the transport across the endoplasmic reticulum membrane as part of the glucose-6-phosphatase complex. However, this protein has not been characterized to any significant degree and, therefore, is not discussed here.

The present review focuses on aspects of the regulation of the so-called insulin-responsive glucose transporter, GLUT4. This transporter has been the subject of intense investigation since the isolation of the corresponding cDNA in the late 1980s, principally driven by the need to understand the mechanism by which insulin regulates glucose disposal into the peripheral tissues, muscle and fat, which are the major sites of expression of this transporter isoform. Moreover, the potential dysfunction of this transporter or its regulatory mechanism(s) are probably aetiological factors in the ontogeny of diabetes mellitus, a major health issue in the Western world. Here we focus on some recent advances in our understanding of the regulation of this transporter, as other recent reviews have provided excellent descriptions of the family of glucose transporters as a whole (Baldwin, 1993; Bell et al. 1993; Gould & Holman, 1993; Mueckler, 1994).

INSULIN-STIMULATED GLUCOSE TRANSPORT IN ADIPOCYTES

Insulin stimulates glucose uptake into isolated adipocytes by up to 30-fold (in the case of rat adipocytes). This increase is mediated by the movement ('translocation') of a large pool of intracellular transporters to the plasma membrane in response to the binding of insulin by its receptor. Adipocytes (brown and white) and muscle are, by virtue of the specific expression of an insulin-regulatable glucose transporter, GLUT4, exquisitely insulin-sensitive. It is the translocation of this transporter isoform which is largely responsible for the 20- to 30-fold increase in glucose transport observed in response to insulin (for review, see Bell et al. 1993; Gould & Holman, 1993; James & Piper, 1994). Interestingly, in resting adipocytes, GLUT4 is effectively sequestered inside the cell until such time as insulin signals its translocation to the plasma membrane, a phenomenon unique to this isoform (see p. 182).

GLUT4 AND INSULIN RESISTANCE

Patients with non-insulin-dependent diabetes mellitus (NIDDM) exhibit reduced rates of insulin-stimulated glucose transport. Given the central importance of GLUT4 in mediating insulin-stimulated glucose transport, the mechanism(s) by which such insulin-resistance may be explained is the subject of intense research effort.

The search for dysfunctional GLUT4 genes

Patients have been examined extensively for mutations of the GLUT4 gene which might result in a dysfunctional transporter. Point mutations have been noted but these occur in too small a proportion of cases to be considered an important aetiological factor in insulin resistance (Choi et al. 1991; Kusari et al. 1991; Buse et al. 1992). Gene defects would be expected to underlie an irreversible form of insulin resistance; however, the phenomenon of insulin resistance appears to be subject to a number of influences, and is not always irreversible (e.g. gestational diabetes mellitus (GDM), see p. 183).

Functional impairment of GLUT4 translocation or reduced expression?

Then, perhaps, it is not surprising that functional impairment of insulin-stimulated glucose transport in NIDDM patients has emerged as being more important than gene
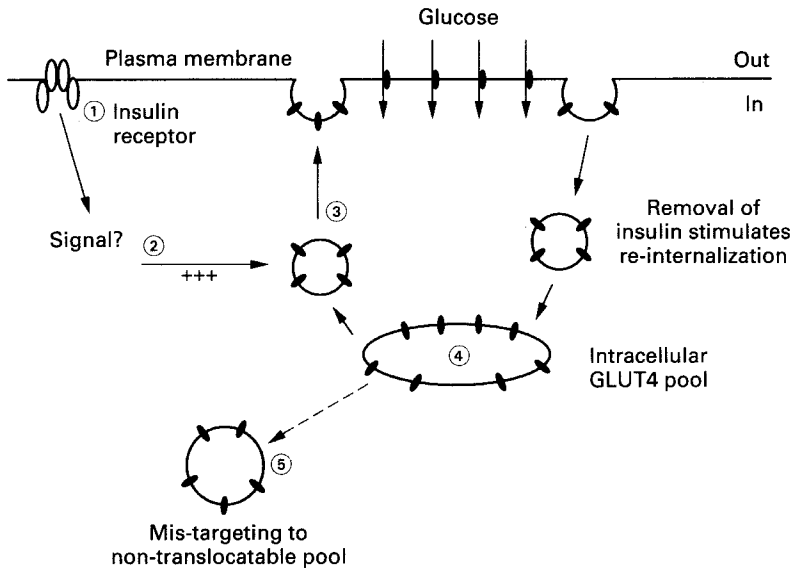
Fig. 1. Insulin-stimulated glucose transport in adipocytes. The insulin-regulatable facilitative glucose trans-porter GLUT4 is expressed in insulin-sensitive tissues, i.e. adipose tissue and skeletal and cardiac muscle. Under basal conditions approximately 95% of GLUT4 is located intracellularly in a pool of vesicles, the nature of which is poorly understood. GLUT4 undergoes a slow rate of constitutive recycling between the plasma membrane and this intracellular site, a process which is thought to occur through coated pits and entry into the endosomal system. On insulin stimulation, 40–50% of the intracellular pool translocates rapidly (within minutes) giving rise to 20–30-fold increases in GLUT4 levels at the cell surface, thus accounting for the large increase in glucose transport observed under such conditions. As circulating glucose and insulin levels fall, there is a reversal of this situation with the cell surface GLUT4 becoming re-sequestered into an intracellular pool. This model is based on studies in adipocytes, the situation in skeletal muscle may differ. Potential sites of insulin resistance shown are: 1, reduced binding of insulin to its plasma membrane insulin receptor or impaired activation of the receptor-associated tyrosine kinase; 2, defective intracellular insulin signalling pathway (defects 1 and 2 would render cells insulin-resistant for glucose transport independently of any defects in transporter expression or function); 3, defective translocation of GLUT4 to the cell surface (in this case, normal levels of GLUT4 are present in the intracellular pool, but a defect in the mechanism responsible for moving this pool to the cell surface results in blunted insulin-stimulated glucose transport); 4, reduction in the intracellular pool of GLUT4 (in this scenario, translocation occurs normally, but there is a reduced level of insulin-stimulated glucose transport as a consequence of a profound reduction in GLUT4 available to be translocated; 5, mis-targeting of GLUT4 to a non-translocatable pool (the mis-localization of GLUT4 to a site from which it cannot be targeted would have the effect of reducing insulin-stimulated glucose transport).

defects. The types of defects found in insulin-regulatable glucose transport can be divided into two groups: (1) situations where there is cellular depletion of GLUT4 and (2) where the protein is apparently expressed at normal levels but fails to translocate effectively to the cell surface in response to insulin, owing to either a defective signal pathway following binding of insulin to its receptor, or potentially as a consequence of mis-targeting to a non-insulin-responsive intracellular site (Fig. 1; for recent review, see Livingstone & Gould, 1995).

In adipose tissue from obese and NIDDM patients there is a clear and well-documented reduction in GLUT4 expression compared with that of lean controls

(Garvey *et al.* 1991); insulin-stimulated GLUT4 translocation is impaired in adipocytes from such individuals (Ciaraldi *et al.* 1991). These patients have lower-than-normal levels of GLUT4 mRNA, indicative of a pretranslational effect. A similar picture is observed in GDM. In a study from Garvey's (Garvey *et al.* 1993) laboratory, GLUT4 levels were noted to be severely depleted in half the GDM patients studied, but in addition all the GDM cohort in this study exhibited abnormal targeting of the protein to an intracellular compartment from which it could not be effectively translocated to the cell surface in response to insulin stimulation.

In skeletal muscle the situation has proved to be different, implying that there are tissue-specific differences in the pathology of insulin resistance as well as in GLUT4 expression. Most studies have shown no change in muscle cellular GLUT4 expression (either mRNA or protein) in insulin-resistant patients (Handberg *et al.* 1990; Pederson *et al.* 1990; Eriksson *et al.* 1992). Only the plasma-membrane-associated GLUT4 was lower, suggesting an abnormality of translocation of the protein in response to insulin. The hypothesis that altered GLUT4 expression probably does not contribute to skeletal muscle insulin resistance was borne out by a recent study of the GLUT4 promoter region in skeletal muscle from NIDDM patients (Bjorbaek *et al.* 1994). Although some genetic variants were found, none of these were considered likely to impair expression of the GLUT4 gene and, therefore, contribute to insulin resistance. The consensus of opinion, therefore, suggests that reduced expression of GLUT4 does not contribute to skeletal muscle insulin resistance and that the defect in this tissue may lie either on the pathway of insulin signalling or transporter translocation.

Studies such as those already described clearly point to several gaps in our knowledge of GLUT4 regulation in the non-diseased state. Research from our laboratory together with that from colleagues in the field relevant to these areas is outlined briefly below.

GLUT4 TRANSLOCATION IN ADIPOCYTES

In an effort to gain some understanding of the defective nature of insulin-stimulated glucose transport in adipocytes, many studies have used isolated primary rat adipocytes or the cultured cell line, 3T3-L1 adipocytes, as model systems for the study of this process. Here, we outline recent research in several key areas which may impinge directly on the aetiology of insulin resistance.

What is the nature of the intracellular pool?

The exact intracellular location of GLUT4 in the basal state and the mechanism by which it reaches the cell surface on insulin stimulation are unclear. Under non-stimulated conditions more than 95% of the GLUT4 is localized to tubular vesicular elements that are found clustered adjacent to endosomal structures, in the trans Golgi network region or in the cytoplasm often close to the plasma membrane. Insulin decreases the level of GLUT4 by 40–50% at each of these locations, suggesting that all these compartments participate in the insulin-regulated recycling of GLUT4 (Slot *et al.* 1991*a,b*).

Many other endosomally-localized membrane proteins, such as the mannose-6-phosphate receptor (Oka *et al.* 1985), the transferrin receptor (TfR; Tanner & Lienhard, 1987, 1989), and GLUT1 (Calderhead & Lienhard, 1988) translocate to the plasma membrane on insulin stimulation in adipocytes; Tanner & Leinhard (1989) were able to

demonstrate that in adipocytes GLUT1, the TfR and the mannose-6-phosphate receptor are co-localized in an intracellular vesicle population. It is now well established that these membrane proteins recycle between an intracellular pool and the plasma membrane, and that this recycling involves the endosomal system (for review, see James & Piper, 1994). Immunoelectron microscopy analysis of GLUT4 in adipocytes has shown that GLUT4 is also located, at least in part, within the endocytic system as it undergoes constitutive recycling through clathrin-coated pits (Slot et al. 1991b; Robinson et al. 1992). Using membrane impermeant, glucose transporter-specific photolabels, Holman and co-workers (Jhun et al. 1992; Satoh et al. 1993) have clearly demonstrated that GLUT4 constantly recycles between the plasma membrane and an intracellular site, both in the presence and absence of insulin, raising the possibility that the intracellular GLUT4 pool may simply comprise elements of the endosomal system.

However, there are several lines of evidence which suggest that GLUT4 trafficking is unlikely to be explained by such a model. First GLUT4 is virtually excluded from the cell surface in non-stimulated cells, whereas this is not the case for other membrane proteins such as GLUT1 or the TfR (Zorzano et al. 1989; Piper et al. 1991). Second, the externalization rate of GLUT4 is slower than that of other recycling proteins such as the TfR in adipocytes (Tanner & Lienhard, 1987; Yang & Holman, 1993), and insulin results in a much larger increase in GLUT4 at the surface compared with other endosomal proteins (Gould & Holman, 1993; James & Piper, 1994). Finally, proteins known to be involved in regulated secretory pathways in other cell types, such as synaptobrevin and secretory-vesicle-associated proteins (SCAMP) have been found in the intracellular GLUT4 vesicles in adipocytes (Cain et al. 1992). These results, together with a mathematical analysis of GLUT4 translocation and recycling in adipocytes (Holman et al. 1994) point to the existence of a unique intracellular pool of GLUT4, which may be considered to be either a sub-endosomal compartment or a separate entity.

We have examined the co-localization of GLUT4 with the TfR, a protein which is known to recycle through the endosomal system (Livingstone et al. 1996). Using a monoclonal antibody specific for a cytoplasmic epitope in GLUT4, we immunoisolated a vesicular fraction from the low-density microsomal fraction of 3T3-L1 adipocytes that contained more than 90% of the immunoreactive GLUT4 found in this fraction, but only 40% of the low-density microsomal TfR. These findings suggest only a limited degree of co-localization of these proteins.

Using a technique to cross-link and render insoluble ('ablate') intracellular compartments containing the TfR by means of a transferrin–horseradish peroxidase (EC 1.11.1.7) conjugate we further examined the relationship between the recycling pathway and the intracellular compartment containing GLUT4 in these cells. We were able to resolve overlapping, but distinct intracellular distributions of the TfR and GLUT4 in murine 3T3-L1 adipocytes (Livingstone et al. 1996). At least three separate compartments were identified: TfR positive (+ve)–GLUT4 negative (−ve), TfR −ve–GLUT4 +ve, and TfR +ve–GLUT4 +ve, as defined by the relative abundance of these two markers. We have proposed that the TfR −ve–GLUT4 +ve compartment, which contains approximately 60% of the intracellular GLUT4, might represent a specialized intracellular compartment that is withdrawn from the endosomal system (Livingstone et al. 1996). So what does this TfR −ve pool of GLUT4 represent? The TfR is considered to be the best marker of early recycling endosomes, therefore, this would preclude this compartment from being endosomally derived, consistent with electron microscopy data

(Slot *et al.* 1991*a,b*). It is noteworthy that a TfR −ve intracellular pool of GLUT4 has also been identified following expression of this transporter in PC12 cells by stable transfection (Herman *et al.* 1994), suggesting that this compartment may not be unique to insulin-sensitive cells.

One possibility that cannot be excluded directly from any of these studies is that this distinct GLUT4 compartment represents trans Golgi reticulum. Slot *et al.* (1991*a,b*) assigned approximately 13% of intracellular GLUT4 to the trans Golgi reticulum in brown adipocytes and a similar amount in cardiac muscle so there is little doubt that GLUT4 does populate this compartment. However, it has recently been shown by vesicle immunoadsorption that TGN38, a putative trans Golgi reticulum marker, is not found in GLUT4 vesicles isolated from adipocytes (Martin *et al.* 1994). One possibility that is certainly supported by morphological studies is that the trans Golgi reticulum is a heterogeneous compartment made up of discrete elements, some of which may be GLUT4 +ve and others that may be TGN38 +ve. Until this complexity has been resolved we cannot exclude trans Golgi reticulum as being a major site for intracellular GLUT4 storage. Similarly, we cannot exclude the possibility that this compartment is derived from late endosomes. However, this does not seem likely as other late endosomal proteins such as the mannose-6-phosphate receptor do not translocate to the cell surface with insulin to the same extent as GLUT4 .

An alternative proposal which is supported by a growing body of evidence is that this non-ablatable GLUT4 pool may be a specialized secretory compartment, analogous to small synaptic vesicles. Evidence in favour of this has been provided from several studies. For example, membrane proteins which are found in regulated secretory compartments in other cell types have been found in the GLUT4-containing compartment in adipocytes (Cain *et al.* 1992; Laurie *et al.* 1993; Südhof *et al.* 1993; James & Piper, 1994). Cain *et al.* (1992) showed that VAMP, a neuronal synaptobrevin homologue may be found in the GLUT4 compartment in adipocytes. In addition, SCAMP, proteins found in all regulated secretory carrier membranes, have also been co-localized with GLUT4 in intracellular membranes (Cain *et al.* 1992; Laurie *et al.* 1993). GLUT4 has also been shown to target the regulated secretory pathway when heterologously expressed in PC12 cells (Hudson *et al.* 1993). These findings argue in favour of the concept of a GLUT4-containing pool which may represent a discrete compartment which has a specialized function to regulate GLUT4 levels at the plasma membrane transiently.

What is the role of cAMP in the regulation of GLUT4 expression and function?

The identification of the factor(s) which controls GLUT4 protein levels in cells represents one of the next hurdles to be cleared in the quest for a full understanding of insulin-regulated glucose transport. One such regulatory effector of GLUT4 transcription has been identified. cAMP is known to have a suppressive effect on the expression of the GLUT4 gene and it has been suggested that disturbances of cAMP metabolism may play a role in the altered GLUT4 expression associated with type II diabetes (Kaestner *et al.* 1991). It is of interest to note that disturbances in cAMP generation have been noted also in animal models of hypertension, a situation which frequently co-exists with insulin resistance. For example, in vascular smooth-muscle cells from the Milan hypertensive rat, changes in the adenylate cyclase (*EC* 4.6.1.1) second

messenger system have been noted which would tend to enhance cAMP generation and, in this tissue, could play a causal role in the pathogenesis of hypertension (Clarke *et al.* 1994). It remains to be seen whether such changes are widespread and also present in insulin-sensitive tissues. If so, it is possible that elevated cAMP levels may contribute to the reduced GLUT4 expression in skeletal muscle of these animals. The recent identification of the GLUT4 promoter and the ability to use transgenic technology to engineer tissue-specific expression of reporter genes should provide key insights into the regulation of this gene.

Does cAMP regulate GLUT4 trafficking?

One aspect of cAMP function which has yet to receive detailed attention is that changes in cellular cAMP levels may also serve to regulate GLUT4 translocation and/or trafficking. It is well established that exposure of adipocytes to insulin followed by treatment with β-agonists such as isoproterenol results in a significant reduction in the rate of glucose transport. This effect is potentially mediated by changes in the trafficking of GLUT4 in the cell (Vannucci *et al.* 1992). Studies have indicated that in response to insulin, the levels of GLUT4 in the plasma membrane of rat adipocytes increase approximately 20- –30-fold. In response to β-agonists both basal and insulin-stimulated transport rates are halved, but in contrast the levels of GLUT4 in the plasma-membrane fractions isolated by differential centrifugation did not decrease (Simpson & Cushman, 1986). Using a membrane-impermeant glucose-transporter-specific probe, Vannucci *et al.* (1992) suggest that the action of β-agonists might be explained by the accumulation of GLUT4 in an occluded vesicle population which is part of the recycling pathway of GLUT4. We are presently addressing this hypothesis using the compartment ablation approach described previously.

However, there is another point which should be considered. James *et al.* (1989) and Lawrence *et al.* (1990) have shown that there is a site within the C-terminus of GLUT4 that contains a serine residue which is a substrate for cAMP-dependent protein kinase (*EC* 2.7.1.37) *in vitro*. Of particular note, this serine residue is immediately adjacent to a di-leucine internalization motif, which is important for the internalization of GLUT4, both when heterologously expressed in fibroblasts or Chinese hamster ovary cells (Czech *et al.* 1993; Corvera *et al.* 1994), and also in adipocytes (C. Livingstone and G. W. Gould, unpublished results). It will be important to determine whether the phosphorylation of this serine residue regulates the recognition of the di-leucine internalization motif and, thus, may regulate glucose-transporter recycling in response to insulin. We are presently engaged on such studies.

What is the signal for translocation generated by the insulin receptor?

Many of the molecular details of the insulin-signalling cascade are now beginning to emerge from the work of many laboratories (for recent review, see White & Kahn, 1994). Within the context of insulin-regulated GLUT4 translocation, however, there is still much to be discovered. As yet, there is no definitive evidence for the precise signal responsible for GLUT4 translocation. It is clear that the tyrosine kinase (*EC* 2.7.1.112) activity of the receptor is required, suggesting that the early events of the pathway probably involve the mediation of SH-2 domain-containing proteins.

Recent data from several laboratories have demonstrated that insulin-stimulated glucose transport may be inhibited by the phosphatidylinositol-3-kinase inhibitor, wortmannin (Okada *et al.* 1994). Moreover, Holman and colleagues (Clarke *et al.* 1994) have demonstrated an effective inhibition of translocation of GLUT4 to the cell surface in response to insulin when wortmannin is present in the bathing media of the cells. This important observation, together with other studies (for example, Gould *et al.* 1994), has strongly implicated phosphatidylinositol-3-kinase in the regulation of glucose transport. What is not clear is how this enzyme impinges on translocation of a membrane protein. One possible explanation has come from work in budding yeast which has clearly shown an important role for this enzyme in the fusion of intracellular transport vesicles, i.e. membrane vesicles which are involved in the intracellular trafficking of membrane proteins and secreted proteins. Thus, phosphatidylinositol-3-kinase may play a role in the regulation of GLUT4 translocation by regulating in some way the membrane trafficking of GLUT4-containing vesicles within the adipocyte. Further studies are required to test this hypothesis.

This explanation of the phosphatidylinositol-3-kinase data is the most plausible, since Birnbaum (Fingar *et al.* 1993) has shown that rapamycin, a specific inhibitor of p70 ribosomal S6 kinase, one of putative down-stream targets of phosphatidylinositol-3-kinase is without effect on insulin-stimulated glucose transport.

One signalling cascade that does not seem to play a role in the regulation of GLUT4 translocation is the mitogen-activated protein (MAP) kinase cascade. Studies from James' laboratory (Robinson *et al.* 1993) have shown that in 3T3-L1 adipocytes both epidermal growth factor (EGF) and insulin stimulate the activity of MAP kinase to a broadly comparable extent. However, only insulin is able to stimulate glycogen synthesis and glucose transport. A similar analysis by Gould *et al.* (1994) strongly suggests that growth factors such as EGF and platelet-derived growth factor stimulate a small increase in glucose transport in adipocytes, but this increase is mediated predominantly via a slight increase in cell surface GLUT1 levels (the adipocyte 'house-keeping' transporter), but only insulin stimulates GLUT4 translocation. These findings suggest that activation of MAP kinase alone is not sufficient to stimulate translocation of GLUT4. However, it would not be prudent to completely rule out a role for this enzyme, as the possible synergistic involvement of MAP kinase with an insulin-specific signal may prove important (Denton & Tavare, 1995).

The recent identification of other MAP kinase homologues and the availability of specific inhibitors of these key kinases will be an area of intense interest and activity over the next few months.

REFERENCES

Baldwin, S. A. (1993). Mammalian passive glucose transporters: members of an ubiquitous family of active and passive transport proteins. *Biochimica et Biophysica Acta* **1154**, 17–49.

Bell, G. I., Burant, C. F., Takeda, J. & Gould, G. W. (1993). Structure and function of mammalian facilitative sugar transporters. *Journal of Biological Chemistry* **268,** 19161–19164.

Bjorbaek, C., Echwald, S. M., Hubrecht, P., Vestegaard, H., Hansen, T., Zierath, J. & Pedersen, O. (1994). Genetic variants in promoter and coding regions of the muscle glycogen synthase and the insulin-responsive GLUT4 genes in NIDDM. *Diabetes* **43,** 976–983.

Brot-Laroche, E. (1996). Differential regulation of the fructose transporters GLUT2 and GLUT5 in the intestinal cell line Caco-2. *Proceedings of the Nutrition Society* **55,** 201–208.

Burant, C. F., Takeda, J., Brot-Laroche, E., Bell, G. I. & Davidson, N. O. (1992). Fructose transporter in human spermatozoa and small intestine is GLUT5. *Journal of Biological Chemistry* **267,** 14523–14526.

Buse, J. B., Yasuda, K., Lay, T. P., Seo, T. S., Karam, J. H., Seino, S. & Bell, G. I. (1992). Human GLUT4/muscle-fat glucose transporter gene: characterisation and genetic variation. *Diabetes* **41,** 1436–1445.

Cain, C. C., Trimble, W. S. & Lienhard, G. E. (1992). Members of the VAMP family of synaptic vesicle proteins are components of glucose transporter containing vesicles from rat adipocytes. *Journal of Biological Chemistry* **267,** 11681–11684.

Calderhead, D. M. & Lienhard, G. E. (1988). Labelling of glucose transporters at the cell surface of 3T3-L1 adipocytes. *Journal of Biological Chemistry* **263,** 12171–12174.

Choi, W.-H., O'Rahilly, S., Buse, J. B., Rees, A., Morgan, R., Flier, J. S. & Moller, D. E. (1991). Molecular scanning of insulin-responsive glucose transporter (GLUT4) gene in NIDDM subjects. *Diabetes* **40,** 1712–1718.

Ciaraldi, T. P., Molina, J. M. & Olefsky, J. M. (1991). Insulin action kinetics in adipocytes from obese and non-insulin dependent diabetes mellitus subjects: identification of multiple cellular defects in glucose transport. *Journal of Clinical Endocrinology and Metabolism* **72,** 876–882.

Clarke, J. F., Young, P. W., Yonezawa, K., Kasuga, M. & Holman, G. D. (1994). Inhibition of the translocation of GLUT1 and GLUT4 in 3T3-L1 cells by the P13 kinase inhibitor wortmannin. *Biochemical Journal* **300,** 631–634.

Corvera, S., Chawla, A., Chakrabati, R., Joly, M., Buxton, J. & Czech, M. P. (1994). A double leucine within the GLUT4 glucose transporter COOH-terminal domain functions as an endocytosis signal. *Journal of Cell Biology* **126,** 979–989.

Czech, M. P., Chawla, A., Woon, C.-W., Buxton, J., Armoni, M., Tang, W., Joly, M. & Corvera, S. (1993). Exofacial epitope-tagged glucose transporter chimeras reveal COOH-terminal sequences governing cellular location. *Journal of Cell Biology* **123,** 127–135.

Denton, R. M. & Tavare, J. M. (1995). Does mitogen-activated protein kinase have a role in insulin action? *European Journal of Biochemistry* **227,** 597–611.

Eriksson, J., Koranyi, L., Bourey, R., Schalinjantti, C., Widen, E., Mueckler, M., Permutt, M. A. & Groop, L. C. (1992). Insulin resistance in type II (non-insulin dependent) diabetic patients and their relatives is not associated with a defect in the expression of the insulin-responsive glucose transporter (GLUT4) gene in skeletal muscle. *Diabetologia* **35,** 143–147.

Fingar, D. C., Hausdorff, S. F., Blenis, J. & Birnbaum, M. J. (1993). Dissociation of pp70 ribosomal protein S6 kinase from insulin-stimulated glucose transport in 3T3-L1 adipocytes. *Journal of Biological Chemistry* **268,** 3005–3008.

Garvey, W. T., Maianu, L., Hueksteadt, T. P., Birnaum, M. J., Molina, J. M. & Ciaraldi, T. P. (1991). Pretranslational suppression of a glucose transporter protein causes insulin resistance in adipocytes from patients with NIDDM and obesity. *Journal of Clinical Investigation* **87,** 1072–1081.

Garvey, W. T., Maianu, L., Zhu, J.-H., Hancock, J. A. & Golichowski, A. M. (1993). Multiple defects in the adipocyte glucose transport system cause cellular insulin resistance in gestational diabetes. *Diabetes* **42,** 1773–1785.

Gould, G. W. & Holman, G. D. (1993). The glucose transporter family: structure, function and tissue-specific expression. *Biochemical Journal* **295,** 329–341.

Gould, G. W., Jess, T. J. H., Andrews, G. C., Herbst, J. J., Plevin, R. J. & Gibbs, E. M. (1994). Evidence for a role of phosphatidylinositol 3-kinase in the regulation of glucose transport in *Xenopus* oocytes. *Journal of Biological Chemistry* **269,** 26622–26625.

Gould, G. W., Merrall, N. W., Martin, S., Jess, T. J., Campbell, I. W., Calderhead, D. C., Gibbs, E. M., Holman, G. D. & Plevin, R. J. (1994). Growth factor induced stimulation of hexose transport in 3T3-L1 adipocytes: evidence that insulin-induced translocation of GLUT4 is independent of activation of MAP kinase. *Cellular Signalling* **6,** 313–320.

Handberg, A., Vaag, A., Damsbo, P., Beck-Nielsen, H. & Vinten, J. (1990). Expression of insulin regulatable glucose transporters in skeletal muscle from type II diabetic patients. *Diabetologia* **33,** 625–627.

Herman, G. A., Bonzelius, F., Cieutat, A.-M. & Kelly, R. B. (1994). Transcriptional repression of the mouse insulin-responsive glucose transporter (GLUT4) gene by cAMP. *Proceedings of the National Academy of Sciences, USA* **91,** 12750–12754.

Holman, G. D., Leggio, L. L. & Cushman, S. W. (1994). Insulin stimulated glucose transporter recycling. *Journal of Biological Chemistry* **269,** 17516–17524.

Hudson, A. W., Fingar, D. C., Seidner, G. A., Griffiths, G., Burke, B. & Birnbaum, M. J. (1993). Targeting of the insulin-responsive glucose transporter to the regulated secretory pathway in PC12 cells. *Journal of Cell Biology* **122,** 579–588.

James, D. E., Hiken, J. F. & Lawrence, J. C. Jr (1989). Isoproterenol stimulates phosphorylation of the insulin-regulatable glucose transporter in rat adipocytes. *Proceedings of the National Academy of Sciences, USA* **86,** 8368–8372.

James, D. E. & Piper, R. C. (1994). Insulin resistance, diabetes and the insulin-regulated trafficking of GLUT4. *Journal of Cell Biology* **126,** 1123–1126.

Jhun, B. H., Rampal, A. L., Liu, H., Lachaal, M. & Jung, C. Y. (1992). Effects of insulin on steady state kinetics of GLUT4 subcellular distribution in rat adipocytes. *Journal of Biological Chemistry* **267,** 17710–17715.

Kaestner, K. H., Flores-Riveros, J. C., McLenithan-Janicot, M. & Lane, M. D. (1991). Transcriptional repression of the mouse GLUT4 gene by cAMP. *Proceedings of the National Academy of Sciences, USA* **88,** 1933–1937.

Kozka, I. J. & Holman, G. D. (1993). Metformin blocks downregulation of cell surface GLUT4 caused by chronic insulin treatment of rat adipocytes. *Diabetes* **42,** 1159–1165.

Kusari, J., Varma, U. S., Buse, J. B., Henry, R. R. & Olefsky, J. M. (1991). Analysis of the gene sequence of the insulin receptor and the insulin-sensitive glucose transporter (GLUT4) in patients with common type II non-insulin dependent diabetes mellitus. *Journal of Clinical Investigation* **88,** 1323–1330.

Laurie, S. M., Cain, C. C., Lienhard, G. E. & Castle, J. D. (1993). The glucose transporter GLUT4 and secretory carrier membrane proteins (SCAMPs) colocalise in rat adipocytes and partially segregate during insulin stimulation. *Journal of Biological Chemistry* **268,** 19110–19117.

Lawrence, J. C. Jr, Hiken, J. F. & James, D. E. (1990). Phosphorylation of the glucose transporter in rat adipocytes. *Journal of Biological Chemistry* **265,** 2324–2332.

Livingstone, C. & Gould, G. W. (1995). Insulin resistance in diabetes mellitus: defects in the insulin-regulatable glucose transporter plays an important role. *Scottish Medical Journal* **40,** 37–39.

Livingstone, C., Hanpeter, D. F., Rice, J. E., James, D. E. & Gould, G. W. (1996). Compartment ablation analysis of adipocyte GLUT4. *EMBO Journal* (In the Press).

Martin, S., Reaves, B., Banting, G. & Gould, G. W. (1994). Analysis of the colocalisation of the insulin-responsive glucose transporter (GLUT4) and the *trans* Golgi network marker TGN38 within 3T3-L1 adipocytes. *Biochemical Journal* **300,** 743–749.

Mueckler, M. (1990). Family of glucose transporter genes: implications for glucose homeostasis and diabetes. *Diabetes* **39,** 6–11.

Oka, Y., Rozek, L. M. & Czech, M. P. (1985). Insulin activates the appearance of insulin-like growth factor II receptors on the adipocyte cell surface. *Journal of Biological Chemistry* **260,** 9435–9442.

Okada, T., Sakuma, L., Fukui, Y., Hazeki, O. & Ui, M. (1994). Essential role of phosphatidylinositol 3′-kinase in induced glucose transport and antilipolysis in rat adipocytes. *Journal of Biological Chemistry* **269,** 3563–3567.

Pedersen, O., Bak, J. F., Anderson, P. H., Lund, S., Moller, D. E., Flier, J. S. & Kahn, B. B. (1990). Evidence against altered expression of GLUT1 or GLUT4 in skeletal muscle of patients with obesity or NIDDM. *Diabetes* **39,** 865–870.

Piper, R. C., Hess, L. J. & James, D. E. (1991). Differential sorting of two glucose transporters expressed in insulin-sensitive cells. *American Journal of Physiology* **260,** C570–C580.

Robinson, L. J., Pang, S., Harris, D. S., Heuser, J. & James, D. E. (1992). Translocation of the glucose transporter GLUT4 to the cell surface in permeabilised adipocytes: effects of ATP, insulin, GTP-γ-S and localisation of GLUT4 to clathrin lattices. *Journal of Cell Biology* **117,** 1181–1196.

Robinson, L. J., Razzack, Z. F., Lawrence, J. D. Jr & James, D. E. (1993). Mitogen-activated protein kinase activation is not sufficient for stimulation of glucose transport or glycogen synthase in 3T3-L1 adipocytes. *Journal of Biological Chemistry* **268,** 26422–26427.

Satoh, S., Gonzalez-Mulero, O. M., Clark, A. E., Kozka, I. J., Quon, M. J., Cushman, S. W. & Holman, G. D. (1993). Use of bismannose photolabels to elucidate insulin-regulated GLUT4 subcellular trafficking kinetics in rat adipose cells: evidence that exocytosis is a critical site of hormone action. *Journal of Biological Chemistry* **268,** 17820–17829.

Simpson, I. A. & Cushman, S. W. (1986). Hormonal regulation of mammalian glucose transport. *Annual Review of Biochemistry* **55**, 1059–1069.

Slot, J. W., Gueze, H. J., Gigengack, S., James, D. E. & Lienhard, G. E. (1991*a*). Translocation of the glucose transporter GLUT4 in cardiac myocytes of the rat. *Proceedings of the National Academy of Sciences, USA* **88**, 7815–7819.

Slot, J. W., Gueze, H. J., Gigengack, S., Lienhard, G. E. & James, D. E. (1991*b*). Immuno-localisation of the insulin-regulatable glucose transporter in brown adipose tissue of the rat. *Journal of Cell Biology* **113**, 123–135.

Südhof, T. C., De Camilli, P., Neimann, H. & Jahn, R. (1993). Membrane fusion machinery: insights from synaptic proteins. *Cell* **75**, 1–4.

Tanner, L. I. & Lienhard, G. E. (1987). Insulin elicits a redistribution of transferrin receptors in 3T3-L1 adipocytes through an increase in the rate constant for receptor externalisation. *Journal of Biological Chemistry* **262**, 8975–8980.

Tanner, L. I. & Lienhard, G. E. (1989). Localisation of transferrin receptors and insulin-like growth factor II receptors in vesicles from 3T3-L1 adipocytes that contain glucose transporters. *Journal of Cell Biology* **108**, 1537–1545.

Vannucci, S. J., Nishimura, H., Satoh, S., Cushman, S. W., Holman, G. D. & Simpson, I. A. (1992). Changes in cell surface accessibility of GLUT4 glucose transporter: effects of insulin, isoproterenol and adenosine. *Biochemical Journal* **288**, 325–330.

Waddell, I. D., Zomerschoe, A. G., Voice, M. W. & Burchell, A. (1992). Cloning and expression of a hepatic microsomal glucose transport protein. *Biochemical Journal* **286**, 173–177.

White, M. F. & Kahn, C. R. (1994). The insulin signalling system. *Journal of Biological Chemistry* **269**, 1–4.

Yang, J. & Holman, G. D. (1992). Comparison of GLUT4 and GLUT1 subcellular trafficking in basal and insulin-stimulated 3T3-L1 cells. *Journal of Biological Chemistry* **268**, 4600–4603.

Zorzano, A., Wilkinson, W., Kotlair, N., Thoidis, G., Wadzinski, B. E., Ruoho, A. E. & Pilch, P. F. (1989). Insulin-regulated glucose uptake in rat adipocytes is mediated by two transporter isoforms present in at least two vesicle populations. *Journal of Biological Chemistry* **264**, 12358–12363.